Molecular Diodes: Irreversible Motion in Nanofabricated Arrays

Forward

This work sprung from my collaboration with Prof. Jim Sturm and his students Richard Huang, David Inglis and John Davis. Richard Huang came up with a brilliant and simple scheme by which a offset array of posts could deterministically separate in a binary manner large objects from small objects with extraordinary precision in a low Reynold number flow. The underlying idea behind this "bump" array is what a physicist grandly calls broken symmetry: the offset row of posts makes bundles of streamlines jitter back and forth, larger objects sample more than one streamline bundle and are deflected along the clear-axis of the offset array.

A straightforward application of the original idea down in size to objects well below a micron is fraught with difficulties because diffusion contravenes the deterministic physics of the bump array, without further new ideas the only way to separate at smaller sizes is to run the flow faster, and at the size of proteins high resolution separation occurs at flow speeds of 1 m/sec and pressure gradients of hundreds of bar/cm: in principle doable but not so interesting.

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Statement of the Problem Studied

We decided to push the technology not by brute force by via two paths: creating hydrodynamic metamaterials which allow us to refocus objects by the construction of hydrodynamics lenses ((work done in collaboration with Prof. Steven Chou in Electrical Engineering and his student Keith Morton), and by exploring the changes in separation process by playing with changes in the shapes of the posts themselves (work done in collaboration with Prof. Jim Sturm and his student Kevin Loutherback). Both these projects have been successful.

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Summary of the Most Important Results

The metamaterial design is summarized in Fig. 1. We were able to show [1] that it is possible to direct particles entrained in a fluid along trajectories much like rays of light in classical optics. A nanostructured, asymmetric post array forms the core hydrodynamic element and is used as a building block to construct *nanofluidic metamaterials* and to demonstrate refractive, focusing and dispersive pathways for flowing beads and cells. The core element is based on the concept of deterministic lateral displacement where particles choose different paths through the asymmetric array based on their size: Particles larger than a critical size are displaced laterally at each row by a post and move along the asymmetric axis at an angle to the flow, while smaller, sub-critical particles move with the flow. We create compound elements with complex particle handling modes by tiling the core element using multiple transformation operations; we show that particle trajectories can be bent at an interface between two elements and that particles can be focused into hydrodynamic jets using a single inlet port. Although particles propagate through these elements in a way that strongly resembles light rays propagating through optical elements, there are unique differences in the paths of our particles as compared to photons. The unusual aspects of these modular, nanofluidic metamaterials form a rich design toolkit for mixing, separating and analyzing cells and functional beads on-chip. As an example of the modular aspect of the metamaterial design, Fig. 2 shows the paths formed by particles in a series of focussing and defoccusing hydrodynamic lenses.

A further application of this hydrodynamic metamaterial design was demonstrated by moving *E. coli* cells from one region (which osmotically stressed the cells) of a bumping region to another one (which lysed the cells), lysing the cells in the second region, followed by separation of the bacterial chromosome from cell contents of the lysed cell [2]. Fig. 3 shows a series of images where this deconstruction and separation of cell contents is done.

Having developed complex metamaterial arrays, we then explored what happens if the posts are no longer circular. Naively you might expect that there is little influence of post shape, but this is dramatically wrong! At this point all we have explored are right-angle triangles, it is entirely possible that other geometries will yield even stranger results.

In the case of the triangles, we have played with the position of the hypotenuse of the right angle triangle with respect to the average flow direction in the array. We have been able to construct a true microfluidic ratchet where the trajectory of particles in a certain size range are not reversed when the sign of the driving force is reversed [3]. Significantly, the path of the particles is deterministic, unlike previous microfluidic ratchets that rely on diffusion. This ratcheting effect is produced by employing triangular rather than the conventionally circular posts in a post array (deterministic lateral displacement array) that selectively displaces particles transported through the array. Using this, we demonstrate a fractionation technique where particles can be separated without any net motion of the fluid. The underlying mechanism of this method is shown to to be an asymmetric fluid velocity distribution through the gap between posts.

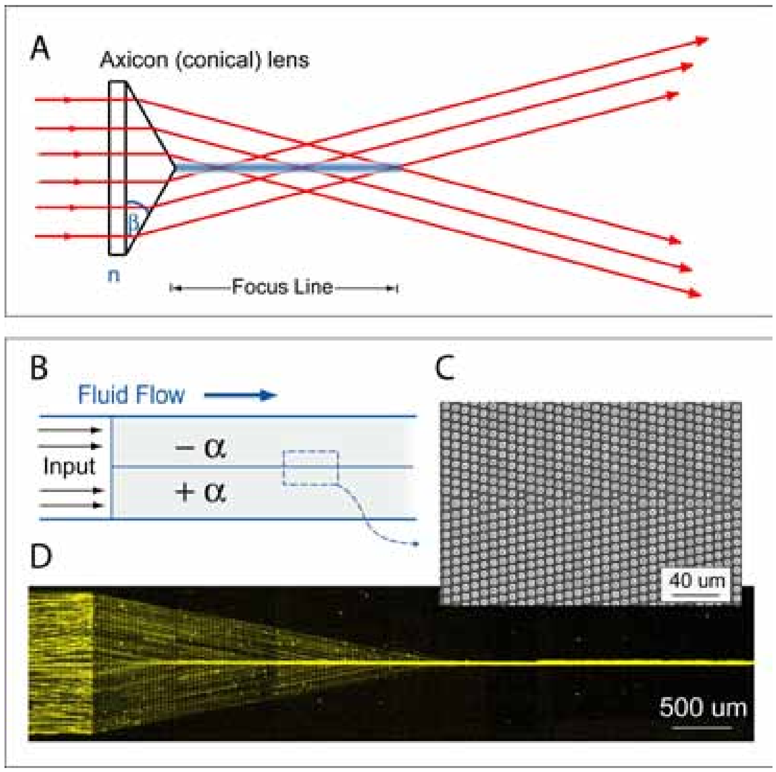

Figure 1: Optical and nanofluidic Focusing Elements. **A** A conical lens or axicon focus collimated incident light into a nominally non-diffracting line. **B** The nanofluidic equivalent of a focusing lens is constructed by tiling a $-\alpha$ array and a $+\alpha$ array vertically. The focusing element $+F$ directs incident particles to a line. **C** SEM image of the interface between the sub-elements. **D** Here 2.7um particles enter the nanofluidic device from a single inlet port and are rapidly focused within a few channel diameters into a continuously flowing hydrodynamic jet.

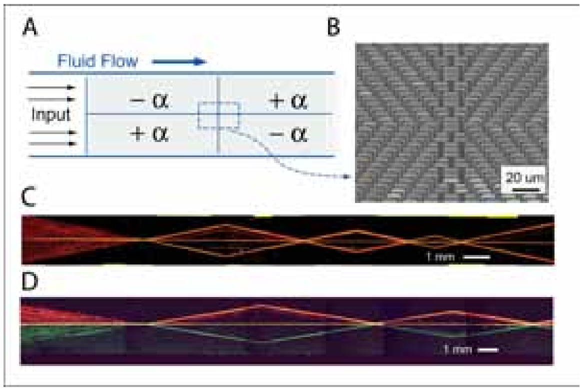

Figure 2: Complex nanofluidic metamaterial. **A** Schematic of a complex metamaterial constructed by tiling several focusing, defocusing, and refractive elements. **B** Tilted, cross-sectional SEM image showing the interface between four sub-elements. **C** Collage of time exposure images showing particle motion through a series of different $+F$ and $-F$ elements, motion is from left to right using just a single inlet and single outlet port. **D**, A similar device with two separate inputs allowing two different-colored bead streams in the top and bottom halves of the device. Observe that particle cross-over between the two halves of the device is rare; particles only mix when dynamically hydrodynamically trapped along the center reflection axis.

The motion of the particles is a true form of an irreversible diode when they are in a certain size range. Typically the posts are round, but we employ triangular posts in the devices discussed here. The bump array consists of array of posts whose lattice is tilted at angle epsilon with respect to the average fluid flow direction (Fig 1). The results of this paper are presented from an array with pitch of 10 microns and a tilt angle ϵ of 5.71° (0.1 radians). Right isosceles triangular posts with 6 microns leg length were used, giving a gap between posts of approximately 4 microns. The array was microfabricated in silicon by reactive-ion etching to a depth of approximately 10 microns and then sealed with a PDMS-coated glass slide.

In such an array, small particles below a critical diameter follow the streamlines of the fluid and travel in the average fluid direction (Fig. 4a Particles larger than the critical diameter are displaced ("bumped")from one streamline to another by the posts and move at an angle ϵ with respect to the average fluid flow. In general, these particle paths are reversible when the fluid flow direction is reversed, as made clear by the time-lapse images of the motion of small (1.1 micron) and large (3.1 micron) fluorescent polystyrene beads when the fluid flow direction was reversed multiple times (Fig. 4(a,b)). The sign of the pressure gradient was switched after the particle had traversed several periods of array to ensure that the observed behavior was characteristic of a particle of that size.) In devices with triangular (as opposed to circular posts used in previous work), we found that particles with an intermediate size (1.9 microns) did not retrace their trajectory when the fluid flow was reversed – rather, they followed the trajectory of large particles in one direction and approximately that of small particles in the other (Fig. 4(c)). When the fluid flow was reversed several times in an oscillatory manner, the net motion of these particles was roughly perpendicular to the axis of oscillatory fluid motion, leading the particles to be separated from their original surrounding plug of fluid without any net motion of the fluid. After many cycles of oscillation, the particles were concentrated along the upper edge of the device and could not be returned to their original

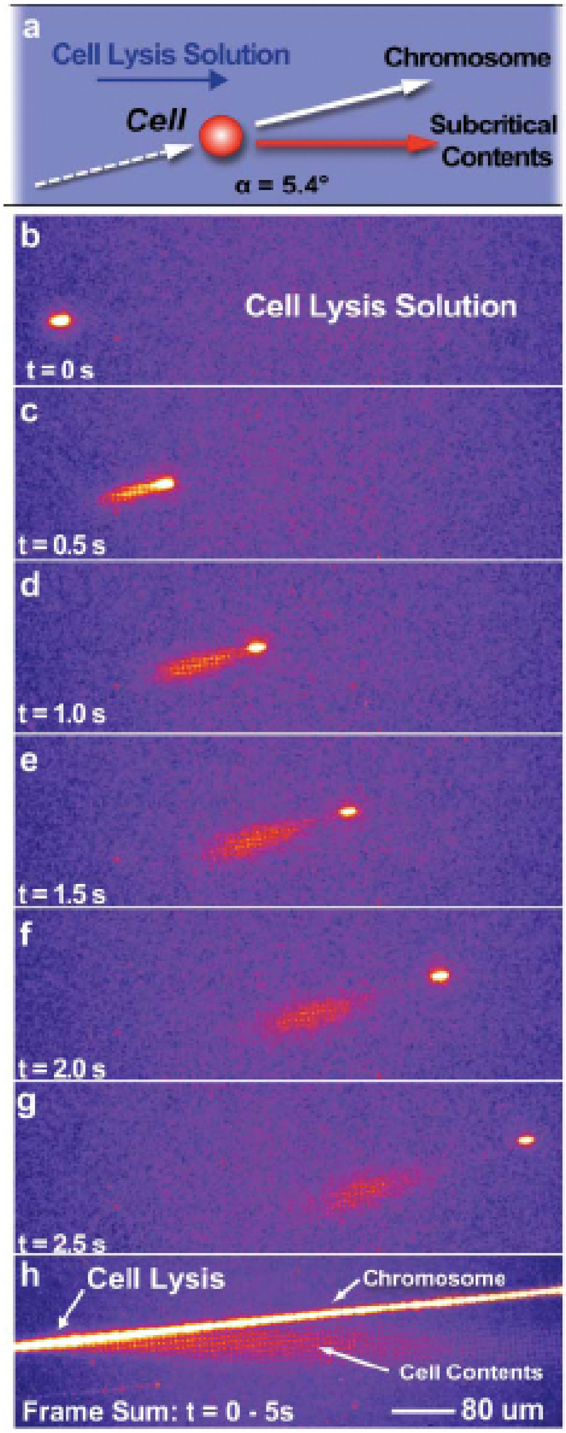

Figure 3: Lysis of a doubly-labeled, spheroplasted E. coli cell as it moves though a lysis solution of 8%SDS. (a) Schematic showing paths taken by various cell components before and after lysis. The chromosome of the GFP-expressing E. coli was fluorescently labeled (Syto13, Invitrogen) to allow tracking of chromosome trajectory in addition to other cellular components, which are visualized by the GFP. Panels (b)(g): Falsecolor time sequence of cell lysis. After lysis, the chromosome continues to track along the bumping trajectory, separating from the rest of the cell lysate which moves straight ahead. Here, color is applied (ImageJ, NIH) to highlight the path differences between the GFP in the cell debris and the brighter chromosome and to draw a distinction with Fig. 3 which showed a GFP tracer stream that is not shown here. Images are 0.5 s apart with a 100 ms shutter speed. Panel (h) shows an open-shutter montage of 5 s of the motion

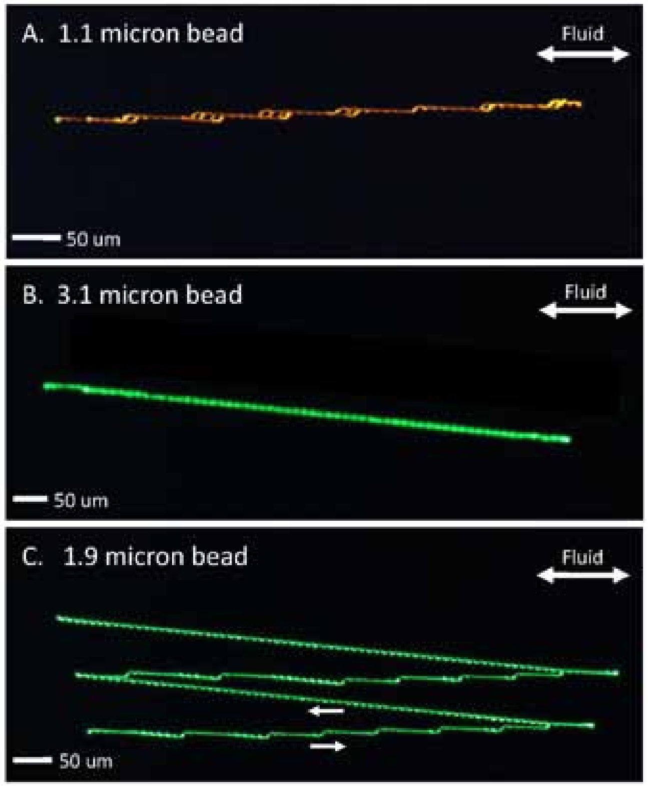

Figure 4: Trajectories of spherical polystyrene beads of three different sizes in an array like that of Fig. 1 as the direction of fluid flow is cycled back and forth twice. Particle sizes are (a) 1.1 microns, (b) 3.1 microns, and (c) 1.9 microns. Particles in (a) & (b) retrace their paths when the direction of the fluid is switched while the trajectory in (c) varies with the direction of the fluid flow. In (c), small arrows indicate the direction of the fluid along the particle path.

positions.

To support this hypothesis, numerical simulations were performed on the array structure used for the experiments using triangular posts, which has $\epsilon = 0.1$, and for a similar array with circular posts. The velocity profiles, normalized for width and peak velocity, were virtually independent of the tilt angle epsilon over a large range of angles (between 1/2 radians to 1/100 radians). The calculated fluid velocity profile across gaps between posts are shown in Fig. 4 for both cases. For round posts, the profile is symmetric about the center of the gap, so there should be no difference in critical particle size for fluid traveling towards the right or left. However, the velocity profile for the triangular post array is biased towards the sharp triangular corner pointing up into the flow stream. In other words, more fluid goes through the lower half of the gap than the top half, and the streamlines are compressed near this vertex. Qualitatively, this means the critical size for a particle to be displaced across a stall line should be smaller when a particle moves along the vertex of the triangle than it would be for the same particle moving along the flat edge of the triangle. Thus, it is possible that a particle can be made to ratchet in a bump array by inducing an asymmetry in the flow profile that results in different critical particle sizes depending on which way the particles are moving through the array.

Conclusion: we have developed an entirely new class of metamaterials, and have extended that work to discover that by making the posts asymmetric a true deterministic ratcheting motion can occur. The next step will be to scale these ideas down below the micron scale.

References

[1] Keith J. Morton, Kevin Loutherback, David W. Inglis, Ophelia K. Tsui, James C. Sturm, Stephen Y. Chou, and Robert H. Austin **Hydrodynamic metamaterials: Microfabricated arrays to steer, refract, and focus streams of biomaterials** PNAS published May 21, 2008, 10.1073/pnas.0712398105

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[3] Loutherback, K., J. Puchalla, R. H. Austin, and J. C. Sturm. 2009. Deterministic Microfluidic Ratchet. Phys Rev Lett 102:10.1103/PhysRevLett.1102.045301